

THE ABOVE HAS BEEN WRITTEN BY DENIS SOLOMON  
PHD, THE SOLE INVENTOR ON FEBRUARY 11<sup>th</sup>, 2002.

\* This was faxed to Fax. no. (703)  
746-9195 Jack in February, 2002.

only Attention : etc. at top of page 1

has been inserted today 11/20/02

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# An *in vitro* examination of an extracellular matrix scaffold for use in wound healing

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describes

**Summary.** This paper provides evidence that an extracellular matrix (ECM) secreted by human umbilical vein endothelial cells (HUVECs)/gelatin assembly overlaid by a mixed matrix secreted by human dermal microvascular endothelial cells (HDMECs) and human dermal fibroblasts provides a viable acellular scaffold for use in wound healing. Trypsinized epidermal keratinocytes or colonies from Dispase-digested fresh and cadaver skin tissue adhered and proliferated on either HUVECs ECM/gelatin or mixed matrix overlaid on HUVECs ECM/gelatin. An epithelial-mesenchymal interaction, previously thought to be tissue-specific, was exposed as well as concomitant integrin versatility. Furthermore, heterologous HDMECs and dermal fibroblasts attached and proliferated on the mixed matrix as well as HUVECs ECM. The conditioned medium from HUVECs (HUVECs CM) was found to neutralize the lingering after effects of Dispase, and could be used for the tissue culture of epidermal keratinocytes, HDMECs and dermal fibroblasts, which share related extracellular secretions. Taken together, these results indicate that cultured epithelial autografts can be redesigned to include both epithelial and dermal elements, and advances the acellular 'sandwich' ECM scaffold as a possible structural replacement for the lamina densa and lamina lucida, damaged or completely missing in some wounds and burns.

**Keywords:** Human skin cell secretions, extracellular matrix scaffold, keratinocyte adhesion, Integrin versatility, cultured epithelial autografts, wound healing

## Introduction

The matrix underlying vascular endothelial cells (ECM) is similar in organization and macromolecular composition

to basement membranes. The macromolecular composition of endothelial cell matrices (ECMs) reportedly includes collagen types I, III, IV, V, VI, fibronectin, laminin, heparan sulphate and dermatan sulphate/chondroitin sulphate proteoglycans, entactin, elastin with sequestered basic fibroblast growth factor (b-FGF), plasminogen activator and heparanase activity (Gospodarowicz *et al.* 1980; Kerner *et al.* 1993).

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**Attention:** 10/076,266 –Filing date 2/15/02

**VOLUNTARY AMENDMENT TO FILED SPECIFICATION**

**'A surgical-medical dressing for the treatment of body burns and for wound healing which employs human umbilical vein endothelial cell conditioned medium for human cell growth used in the manufacture of the dressing'.**

A United Kingdom (U.K.) patent application (GB 0008079.6) which will be published in England on February 13<sup>th</sup>, 2002. The report by the official examiner at the U.K. Patent Office is enclosed.

**CORRECTION**

THE ABOVE LINE SHOULD READ..... **'BASED ON A UNITED KINGDOM (U.K.) patent application (GB 0008079.6)' etc..**

**Differences between U.K. and USA specifications.**

- (1) Different titles of specifications—U.K. reads: 'A

**surgical-medical dressing for the treatment of  
body burns and for wound healing’.**

**USA reads:**

**‘A surgical-medical dressing for the treatment of  
body burns and for wound healing which  
employs human umbilical vein endothelial cell  
conditioned medium for human cell growth used  
in the manufacture of the dressing’.**

- (2) U.K. specification does not state the culture  
medium used to grow the cells----- in the title of  
the specification or in the description of the  
invention.**
- (3) Different claims are stated in support of the two  
different specifications which however do  
contain similarity in certain areas.**

**NO PRIORITY DATE CLAIMED SINCE SPECIFICATIONS  
ARE SUFFICIENTLY DIFFERENT.**

**FILED AS NON-PROVISIONAL (SMALL ENTITY STATUS)  
-UTILITY PATENT.**

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RETURN RECEIPT REQUESTED HAS NOT ARRIVED  
FROM YOUR OFFICE. THEREFORE I HAVE NO FILING  
NUMBER OR FILING DATE TO REFER TO.

**HISTORICAL NOTE**

PLEASE NOTE THAT A PROVISIONAL PATENT (filed  
with your office) WAS GIVEN A FILING DATE OF 11/18/98  
AND AN APPLICATION NUMBER 60/108,836.

YOUR OFFICE GRANTED A FOREIGN FILING LICENCE  
ON 12/16/98 \*\* Small Entity \*\*

TITLE: 'Surgical-medical dressing for the treatment  
body burns and for wound healing'

1<sup>st</sup> U.K. patent given a filing date of 1/28/99 and an  
application number GB 9901773.3

\*\*\*\*\*This application was discarded.\*\*\*\*\*

Amended U.K. patent given a filing date of 4/4/00 and an  
Application Number GB 0008079.6 The first examination  
of this application was paid for on 4/4/01.